Exploring the Effect of Sex on Empirical Fitness Landscapes

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Online enhancements: appendixes.

ABSTRACT: The nature of epistasis has important consequences for the evolutionary significance of sex and recombination. Recent efforts to find negative epistasis as a source of negative linkage disequilibrium and associated long-term advantage to sex have yielded little support. Sign epistasis, where the sign of the fitness effects of alleles varies across genetic backgrounds, is responsible for the ruggedness of the fitness landscape, with several unexplored implications for the evolution of sex. Here, we describe fitness landscapes for two sets of strains of the asexual fungus *Aspergillus niger* involving all combinations of five mutations. We find that ~30% of the single-mutation fitness effects are positive despite their negative effect in the wild-type strain and that several local fitness maxima and minima are present. We then compare adaptation of sexual and asexual populations on these empirical fitness landscapes by using simulations. The results show a general disadvantage of sex on these rugged landscapes, caused by the breakdown by recombination of genotypes on fitness peaks. Sex facilitates movement to the global peak only for some parameter values on one landscape, indicating its dependence on the landscape's topography. We discuss possible reasons for the discrepancy between our results and the reports of faster adaptation of sexual populations.

Keywords: evolution of sex, sign epistasis, fitness landscape, recombination.

Introduction

The way genes interact in their effect on a phenotype or fitness, called epistasis, has important implications for evolution, including the evolution of sex and recombination (Wolf et al. 2000). Epistasis entails any deviation from independent (i.e., additive or multiplicative, depending on the evolutionary model used) gene effects and hence encompasses many different forms of gene interaction. At least two forms of epistasis are relevant for the question of why sex and recombination are evolutionarily maintained. The first is a one-dimensional type of magnitude epistasis, called negative epistasis, where the fitness effects of alleles all have the same sign (i.e., all are either deleterious or beneficial) and the fitness of genotypes with many alleles of the same sign is lower than expected from the product of their individual effects (Barton 1995; Otto and Lenormand 2002). Negative epistasis provides a long-term advantage to sex and recombination by causing negative linkage disequilibria of alleles affecting fitness (Barton 1995). By breaking down negative linkage disequilibria and increasing fitness variation, sex may facilitate the response to selection due to the net production of genotypes carrying extreme numbers of fitness alleles. Besides negative epistasis, genetic drift combined with directional selection can cause negative linkage disequilibria (Hill and Robertson 1966; Felsenstein 1974; Barton 1995; Otto and Lenormand 2002; de Visser and Elena 2007). However, magnitude epistasis also has consequences for fitness in the next generation, and it is the balance between short- and long-term effects of sex that determines the conditions favoring selection for sex and recombination (Barton 1995; Otto and Lenormand 2002). Most experimental studies have concentrated on detecting magnitude epistasis, but despite increasing efforts during the past decade, the support for negative epistasis is limited (de Visser and Elena 2007; Kouyos et al. 2007).

The second type of epistasis with potential implications for the evolution of sex is sign epistasis, where the sign of the fitness effects of alleles varies across genetic backgrounds (Weinreich et al. 2005). Sign epistasis causes adaptive constraints by limiting the number of mutational pathways that can be taken by natural selection. If sign epistasis is locally consistent, such that all mutational pathways leading to a particular genotype show the same fitness change on approach of that genotype, these constraints are most severe and cause local fitness peaks and valleys (Weinreich et al. 2005).

The effect of sex on rugged fitness landscapes is likely to depend on the landscape's topography and thus is largely an empirical problem. Unlike tests of negative epistasis, which are possible with phenotypic analyses (Kouyos et al. 2007), conclusive tests of sign epistasis require knowledge of the genotypes involved. Moreover, to study evolutionary constraints from sign epistasis, a systematic and detailed description of the fitness landscape is needed, re-

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quiring the construction and fitness measurement of many genotypes. These requirements clearly constrain empirical studies of fitness landscapes. However, with the advent of genomic techniques, recent studies have begun to tackle this problem and found ample support for sign epistasis (Weinreich et al. 2005, 2006; Poelwijk et al. 2007; M. Salverda, F. Debets, J. van der Oost, R. Hoekstra, and A. de Visser, unpublished manuscript). Indirect support for fitness landscapes with multiple peaks has come from the analyses of fitness trajectories of evolution experiments with microorganisms, where replicate populations sometimes approach different fitness maxima (Korona et al. 1994; Burch and Chao 2000; Buckling et al. 2003; Schoustra et al. 2007; Rozen et al. 2008).

The evolutionary effects of recombination in the presence of sign epistasis have been addressed mostly in a two-locus setting, where the generic situation is that of two fitness peaks separated by intermediate valley genotypes of lower fitness (Crow and Kimura 1965; Eshel and Feldman 1970; Michalakis and Slatkin 1996; Phillips 1996; Weinreich and Chao 2005). These studies have typically found that recombination slows the transition from the initial (lower) peak to the final (higher) peak to the extent that under certain assumptions this transition can be completely suppressed. Multilocus models have been considered extensively in the realm of evolutionary computation (Reeves and Rowe 2003), where it is well appreciated that the advantage or disadvantage of recombination as a search strategy depends on the structure of the fitness landscape of the problem considered (Manderick et al. 1991). There are a few theoretical studies of recombination in a multilocus fitness landscape by evolutionary biologists (Otto et al. 1994; Bergman et al. 1995; Kondrashov and Kondrashov 2001; Hadany and Beker 2003; Watson and Wakeley 2005), which we will return to in "Discussion."

The aim of this article is twofold. We first analyze fitness data of the filamentous fungus *Aspergillus niger* in order to detect and quantify the occurrence of sign epistasis and multiple fitness peaks, and then we explore the effects of sex on these empirical landscapes by using simulations. The data involve two collections of strains that each carry all possible combinations of five phenotypic marker mutations with individually deleterious effects (four of which are shared by the two collections). These data were collected and analyzed previously to test for prevailing negative epistasis among deleterious mutations, for which we found no significant support (de Visser et al. 1997). Our new analyses indicate the presence of sign epistasis: depending on the genetic background, we find sometimes negative and sometimes positive fitness changes with the addition of the same mutation. We also find local fitness maxima and minima in both data sets, leading to severe adaptive constraints. We then use simulations to compare adaptation of sexual and asexual populations on these empirical rugged fitness landscapes. We find that populations always get stuck on local peaks, from which they may later escape via the production of genotypes containing multiple mutations. The simulation results suggest that sex is disadvantageous under most conditions by breaking down escape genotypes. Only on one landscape and for intermediate recombination rates does sex facilitate the escape from a local peak to the global peak, hence indicating that this benefit depends on the landscape's topography. We discuss these findings in the light of experimental evidence for benefits of sex during adaptation.

Methods

Strain Construction

Aspergillus niger is an asexual filamentous fungus with a predominantly haploid life cycle. A detailed description of the construction of the *A. niger* strains used in this study is given by de Visser et al. (1997). Briefly, we exploited the parasexual cycle of this fungus in order to isolate haploid segregants from a spontaneous heterozygous diploid strain that was isolated from a heterokaryon involving a wild-type strain and a strain containing a single marker mutation on each of its eight chromosomes. Both the wild-type strain and the eight-marker strain contained a spore-color marker on the first chromosome (*olvA1* and *fwnA1*, causing olive- and fawn-colored conidiospores, respectively), helpful for the isolation of haploid segregants from the diploid mycelium, which produced black spores. The seven marker mutations on the other chromosomes include, in increasing chromosomal order, *argH12* (arginine deficiency), *pyrA5* (pyrimidine deficiency), *leuA1* (leucine deficiency), *pheA1* (phenyl-alanine deficiency), *lysD25* (lysine deficiency), *oliC2* (oligomycin resistance), and *crnB12* (chlorate resistance). These marker mutations were arbitrarily chosen, and the only requirement was their phenotypic detectability. They were individually introduced using low-dose UV induction and combined using the parasexual cycle to minimize the probability of introducing additional mutations. Because the wild-type strain and the eight-marker strain had a recent common ancestor, it was unlikely that the segregants differed at more loci than those carrying the marker mutation.

From the $2^8 = 256$ theoretically possible different haploid segregants, 186 were isolated after forced haploidization on medium containing benomyl from among ~2,500 strains tested. Among those strains, two sets of 32 strains each were present that carried all 32 possible combinations of five markers: the wild type (no mutations), the five strains with a single mutation, the 10 double mutants, the 10 triple mutants, the five quadruple mutants,

and the single quintuple mutant. One complete subset, referred to as CS I, involves all combinations of *arg*$^-$, *pyr*$^-$, *leu*$^-$, *oli*^{R}, and *crn*^{R}; CS II contains all possible combinations of *arg*$^-$, *pyr*$^-$, *leu*$^-$, *phe*$^-$, and *oli*$^-$ (both in increasing chromosomal order). Hence, these two complete subsets share four of the five marker mutations involved and are not independent. These complete subsets cover all intermediates between the wild-type strain and the quintuple mutant and thus allow a complete description of the fitness landscape involved (Weinreich et al. 2006).

Fitness Assay

Previously (de Visser et al. 1997), we used the increase in mycelial surface area per unit time on supplemented medium as a fitness estimate for these strains because this measure showed a strong positive correlation with the rate of spore production, which is an intuitive measure of fungal fitness (Pringle and Taylor 2002). There, we used deviations from additivity at the level of log(surface area growth rate) as a measure of epistasis. Here, we use the rate of linear expansion of the radius of a colony spreading from a central source to estimate fitness because it is believed to be a better estimator of the intrinsic growth rate (i.e., unhindered by competition) and because it is constant in time, which makes departure from additive mutational effects a simple and appropriate way to detect epistasis. However, radial growth rate and log(surface area growth rate) give almost identical results because if a linear model is used, variation in one fitness measure explains ~99.5% of the variation in the other; the remaining ~0.5% is explained by the slight curvature of the relationship. Moreover, many of our analyses require only a rank order of fitness values and hence are insensitive to these small differences.

The medium used for the fitness assay is a minimal agar medium supplemented with all amino acids and nitrogen sources required by some of the auxotrophic strains (de Visser et al. 1997). The medium was prepared as a single batch, and 9-cm petri dishes were filled with 20 mL medium using a calibrated pump. Two replicate plates were inoculated in the center with spores from a pregrowth culture of each strain by using a platinum needle. Plates were randomized and incubated at 26°C in the dark for 12 days. Colony diameter was measured after 3 and 12 days in two perpendicular directions, yielding a single estimate of the radial growth rate per replica plate by dividing the average difference in diameter in the two perpendicular directions at these two time points by twice (to derive radius from diameter) the time elapsed. All fitness estimates shown are expressed relative to that of the wild type. The fitness values shown in table 1 are obtained by averaging over the two replicates; the underlying fitness estimates for the individual replicates can be found in table A1 in the online edition of the *American Naturalist*.

Simulations

For the simulations of sexual and asexual populations, we use the Wright-Fisher model (Fisher 1930; Wright 1931) with a fixed number of haploid individuals N. The sequence length of genotypes (i.e., number of loci with two alleles each) is denoted by L, which is 5 for the *A. niger* landscapes, resulting in $2^5 = 32$ haploid genotypes. The algorithm to be employed for recombination is similar to that described by Kim and Orr (2005).

Let the frequency of the genotype σ at generation t be denoted by $f(\sigma; t)$. At first, the population evolves deterministically in the order of selection, mutation, and recombination. After selection and mutation, the frequency of the genotype σ will be

$$p_1(\sigma) = \sum_{\sigma'} \mu_{\sigma\sigma'} \frac{w(\sigma')}{\bar{w}(t)} f(\sigma'; t), \tag{1}$$

where $\bar{w}(t) \equiv \sum_\sigma f(\sigma; t) w(\sigma)$ is the mean fitness of the population at generation t and $\mu_{\sigma\sigma'}$ is the probability that the genotype σ' will be the genotype σ after mutation (if no mutation occurs, $\sigma = \sigma'$). In what follows, we use a mutation scheme such that

$$\mu_{\sigma\sigma'} = (1 - U)\delta_{\sigma\sigma'} + Z_{\sigma\sigma'} \frac{U}{L}, \tag{2}$$

where $\delta_{\sigma\sigma'}$ is the Kronecker delta, which takes the value 1 if $\sigma = \sigma'$ and 0 otherwise, and $Z_{\sigma\sigma'}$ is 1 if the Hamming distance between σ and σ' is 1 and 0 otherwise. This mutation scheme implies that a genotype mutates with probability U and that each change occurs only at one locus, chosen at random on the sequence. When $U \ll 1$, which is the regime we are interested in, this mutation scheme is essentially equivalent to allowing independent mutations at all loci with probability μ ($= U/L$) because the probability of having multiple mutations in one generation is $O(U^2)$, which is negligible compared with the probability of the occurrence of a single mutation.

For the recombination step, we introduce $W_{\sigma|\sigma'\sigma''}$, which is the conditional probability that the resulting sequence is σ in the case that two sequences σ' and σ'' recombine. To be specific, we apply a uniform crossover scheme (Sy-

Table 1: Analysis of local and global fitness maxima and minima on both *Aspergillus niger* landscapes

			CS I			CS II		
Strain	Genotype[a]	Neighbors[b]	Fitness[c]	Rank	Max/min[d]	Fitness[c]	Rank	Max/min[d]
1	00000	2, 3, 4, 5, 6	1	1	**Max**	1	1	**Max**
2	10000	1, 7, 8, 9, 10	.878	3		.878	6	
3	01000	1, 7, 11, 12, 13	.835	10		.835	13	
4	00100	1, 8, 11, 14, 15	.870	5		.870	7	
5	00010	1, 9, 12, 14, 16	.772	20		.909	4	
6	00001	1, 10, 13, 15, 16	.793	16	Min	.772	21	
7	11000	2, 3, 17, 18, 19	.865	6		.865	8	
8	10100	2, 4, 17, 20, 21	.854	8		.854	11	
9	10010	2, 5, 18, 20, 22	.773	19		.923	3	
10	10001	2, 6, 19, 21, 22	.873	4		.773	20	
11	01100	3, 4, 17, 23, 24	.816	14		.816	16	
12	01010	3, 5, 18, 23, 25	.716	24		.852	12	
13	01001	3, 6, 19, 24, 25	.848	9	Max	.716	26	
14	00110	4, 5, 20, 23, 26	.778	18		.855	10	
15	00101	4, 6, 21, 24, 26	.820	12		.778	19	
16	00011	5, 6, 22, 25, 26	.972	2	**Max**	.785	18	
17	11100	7, 8, 11, 27, 28	.816	13		.816	15	
18	11010	7, 9, 12, 27, 29	.748	23		.879	5	
19	11001	7, 10, 13, 28, 29	.832	11		.748	23	
20	10110	8, 9, 14, 27, 30	.749	22		.942	2	Max
21	10101	8, 10, 15, 28, 30	.792	17		.749	22	
22	10011	9, 10, 16, 29, 30	.753	21		.795	17	
23	01110	11, 12, 14, 27, 31	.617	32	Min	.858	9	Max
24	01101	11, 13, 15, 28, 31	.810	15		.617	32	**Min**
25	01011	12, 13, 16, 29, 31	.643	31	Min	.724	25	
26	00111	14, 15, 16, 30, 31	.671	27		.745	24	
27	11110	17, 18, 20, 23, 32	.690	26		.825	14	
28	11101	17, 19, 21, 24, 32	.855	7	Max	.690	27	
29	11011	18, 19, 22, 25, 32	.649	28		.665	29	
30	10111	20, 21, 22, 26, 32	.692	25		.686	28	
31	01111	23, 24, 25, 26, 32	.643	30		.640	30	
32	11111	27, 28, 29, 30, 31	.645	29		.622	31	Min

[a] 0 and 1 indicate the absence or presence of a mutation in chromosomal order: *arg*, *pyr*, *leu*, *oli*, and *crn* for the CS I landscape and *arg*, *pyr*, *leu*, *phe*, and *oli* for the CS II landscape.

[b] Neighbors are genotypes (strain numbers) that differ at a single locus.

[c] Mean relative fitness w, estimated by the ratio of the mycelium growth rates of mutant strain and wild type, where the growth rate of the wild type is 2.41 mm/day.

[d] "Max" and "min" indicate the presence of local fitness maxima or minima, with the global maximum and minimum of each landscape underlined; significant fitness maxima tested with two-sample t-tests between the maximum and each of its five neighbors, using sequential Bonferroni correction, are indicated in bold.

werda 1989) with probability r per replication event.[1] In this case, $W_{\sigma|\sigma'\sigma''}$ takes the form (Boerlijst et al. 1996)

$$W_{\sigma|\sigma'\sigma''} = (1 - \delta_{\sigma'\sigma''}) \times \left[(\delta_{\sigma\sigma'} + \delta_{\sigma\sigma''})\frac{1-r}{2} + F_{\sigma|\sigma'\sigma''}\frac{r}{2^{d(\sigma',\sigma'')}}\right] + \delta_{\sigma\sigma'}\delta_{\sigma\sigma''}, \quad (3)$$

where $d(\sigma', \sigma'')$ is the Hamming distance between the two sequences in the argument and $F_{\sigma|\sigma'\sigma''}$ is 1 if σ can be a recombinant of σ' and σ'' and 0 otherwise. The total num-

[1] Our definition of r differs slightly from the standard notation, where r_{standard} denotes the probability of recombination between adjacent loci (Bürger 2000). In particular, the limit of free recombination in a two-locus system corresponds to $r = 1$, but $r_{\text{standard}} = 1/2$ because recombination occurs in only half of the four possible recombinants, which are created with equal probability according to equation (3). The advantage of our formulation (eq. [3]) is that it provides a compact expression valid for an arbitrary number of loci.

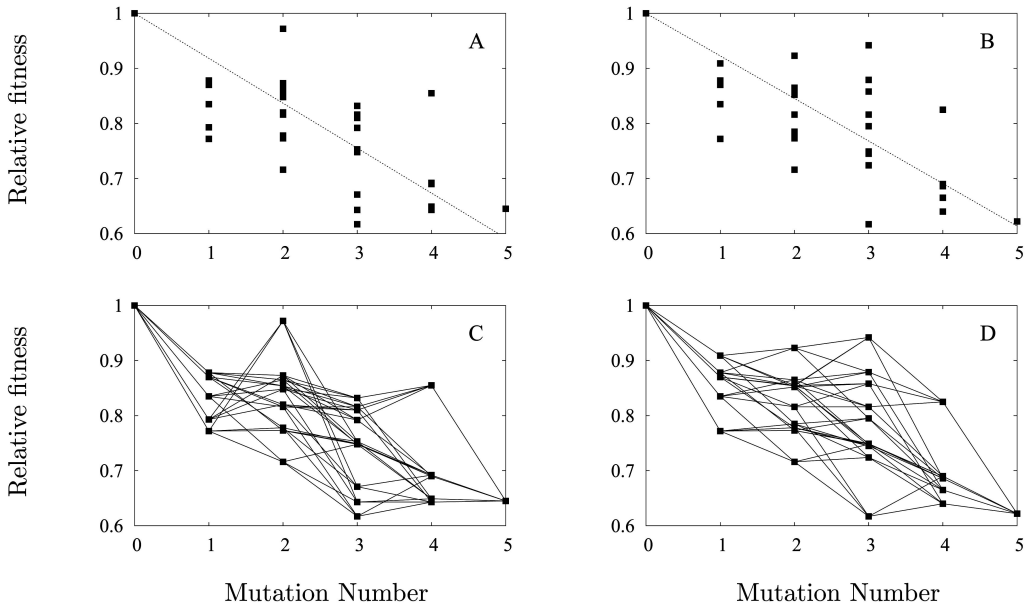

Figure 1: Relative fitness versus mutation number for both complete subsets (CS) of 32 strains of *Aspergillus niger*; these include *arg*, *pyr*, *leu*, *oli*, and *crn* for CS I and *arg*, *pyr*, *leu*, *phe*, and *oli* for CS II. The overall relationship between fitness and mutation number is best described by a linear model (*dotted line*), both for CS I (*A*) and for CS II (*B*). A model including a quadratic term does not improve the fit significantly, suggesting the absence of a prevailing form of magnitude epistasis. When the 120 possible (direct, i.e., involving five mutations) pathways between the wild-type strain and the quintuple mutant are considered, mutations have a negative or positive effect, depending on the genetic background, both for CS I (*C*) and for CS II (*D*), indicating sign epistasis. The lines connect genotypes that differ at a single locus (i.e., Hamming distance = 1).

ber of different recombinants that can be generated by a given pair is $2^{d(\sigma', \sigma'')}$. For example, the possible recombinants of two sequences 11101 and 10100 by uniform crossover are 10100, 10101, 11100, and 11101, each of which occurs with equal probability according to equation (3). When $d(\sigma', \sigma'') = 0$ or $d(\sigma', \sigma'') = 1$, no new genotypes are generated, and the outcome of the replication event is σ' or σ'', independent of r. For later purposes we note the relation (Boerlijst et al. 1996)

$$d(\sigma, \sigma') + d(\sigma, \sigma'') = d(\sigma', \sigma''), \tag{4}$$

which holds whenever $F_{\sigma|\sigma'\sigma''} = 1$.

Finally, the frequency of each genotype after selection, mutation, and recombination becomes

$$\begin{aligned} \Pr(\sigma) &= \sum_{\sigma', \sigma''} W_{\sigma|\sigma'\sigma''} p_1(\sigma') p_1(\sigma'') \\ &= p_1(\sigma)^2 + 2 \sum_{\langle \sigma', \sigma'' \rangle} W_{\sigma|\sigma'\sigma''} p_1(\sigma') p_1(\sigma''), \end{aligned} \tag{5}$$

where $\langle \sigma', \sigma'' \rangle$ signifies that the sum runs over all pairs of distinct haploid genotypes. The actual population at generation $t + 1$ is now formed by sampling N individuals according to the multinomial distribution with probability $\Pr(\sigma)$.

Results

No Prevailing Magnitude Epistasis

Figure 1*A*, 1*B* shows the relationship between relative fitness and mutation number for both complete subsets of *Aspergillus niger* strains. Using surface area growth rate as a measure of fitness and regressing log fitness against mutation number, we previously found no evidence for prevailing magnitude epistasis because adding a quadratic term to the linear regression model did not significantly improve the fit (de Visser et al. 1997). Using radial growth rate (RGR) to test for departure from additivity by regressing RGR against mutation number confirms this conclusion. For both complete subsets of 32 strains, a linear model explains most of the variation in RGR ($r^2 = 0.991$ and 0.992 and the linear coefficient $\alpha = -0.0816$ and -0.0773 for CS I and CS II, respectively; see fig. 1*A*, 1*B*). In fact, a linear model explains more variation of the present fitness measure than it did for log fitness in our previous analyses (for which we found $r^2 = 0.888$ and 0.881 for CS I and CS II, respectively), confirming that RGR is likely a more sensitive measure for detecting epistasis as a departure from additivity. As before, when added to the model, a quadratic term is estimated to be positive ($\beta = 0.0102$ and 0.0069 for CS I and CS II, respectively),

indicating positive epistasis, but approaches significance only for CS I ($F_{1,30} = 4.135$, $P = .0509$) and not for CS II ($F_{1,30} = 1.850$, $P = .184$). Hence, this analysis fails to reveal significant magnitude epistasis in both data sets.

Mutational Pathways Reveal Sign Epistasis

Our previous analyses comparing the fitness of double mutants with the average of both single mutants revealed the presence of both negative and positive epistasis, with the majority of combinations showing positive epistasis (de Visser et al. 1997); the latter is probably a consequence of the relatively large drop in fitness due to the first mutation, compared with the overall linear trend in figure 1. We determined only whether the fitness of double mutants was higher (indicating positive epistasis) or lower (indicating negative epistasis) than expected from the fitness of both single mutants and did not look for sign epistasis. Here, we seek to reanalyze both complete subsets of 32 *A. niger* strains in order to detect sign epistasis. To do so, we first analyze the $5! = 120$ possible pathways of five mutations connecting the wild-type strain and the quintuple mutant to see whether the addition of some mutations causes fitness to increase rather than decrease. Figure 1*C*, 1*D* shows these mutational pathways for both complete subsets. Almost 80% of the 120 pathways exhibit one or more mutational steps causing fitness to increase (95 in CS I and 93 in CS II). Because all mutations are individually deleterious in the background of the wild-type strain, these fitness reversals indicate sign epistasis among the mutations involved.

To formally demonstrate sign epistasis, we need to show the statistical significance of the observed fitness reversals. Each complete subset contains 80 unique single-mutation steps, and both subsets combined have 128 unique single-mutation steps (not 160, because four of the five mutations are shared). Using conventional Bonferroni correction with a cutoff P value of .05 for all 128 tests combined renders none of the fitness differences significant. Linear regression and ANOVA, however, show that genetic differences between these strains are highly significant when tested collectively against the measurement error and explain almost 95% of the fitness variation within each complete subset (de Visser et al. 1997). The lack of significance of individual tests is therefore due to low statistical power and not to a real absence of fitness differences and is caused by a combination of low replication of fitness assays (which was necessary given that both subsets were part of a much greater collection of strains that were assayed) and the high number of tests. One possible solution is to use a P value of .05 for each individual test and accept that this leads to about six ($=128 \times 0.05$) false positives in both data sets combined. Using this criterion, we find 40 single-mutation steps with a significant fitness effect: 35 declines and five increases. Another possibility is to use a P value corresponding to a minimal false discovery rate, that is, the lowest fraction, i, of false positives among the test results called significant (Storey and Tibshirani 2003). Using this criterion, we find the minimum q to be 0.035 (corresponding to $4.5 = 128 \times 0.035$ false positives), which occurs at a P value of .0117, at which point 19 significant single-mutation steps are identified, including one causing a fitness increase.

The A. niger *Fitness Landscapes Contain Local Maxima and Minima*

Sign epistasis may lead to local fitness maxima surrounded by genotypes of lower fitness and to local fitness minima surrounded by genotypes of higher fitness. We analyzed both data sets in order to detect local fitness maxima and minima by comparing the fitness rank of all 32 genotypes with that of their five single-mutation neighbors. This analysis shows several features that indicate the ruggedness of these fitness landscapes (table 1; fig. 2). First, the wild-type strain represents the global maximum in both landscapes, but the quintuple mutant does not coincide with the global minimum in either landscape; both data sets contain a genotype with lower fitness. Second, in total four maxima and three minima are identified in CS I and three maxima and two minima in CS II (see fig. 2*C*, 2*D*).

To provide statistical support for the presence of local fitness maxima and minima in the two landscapes, we performed two analyses. First, to test whether these fitness maxima and minima differ significantly from each of their five mutational neighbors at Hamming distance 1, we performed two-sample t-tests with sequential Bonferroni correction (Rice 1989). These showed that both the global maximum and the local maximum 00011 of CS I are significant, while in CS II only the global maximum is significantly more fit than all its mutational neighbors (see table 1). Second, to explore the likelihood of multiple fitness maxima and minima, we generated 10^7 resampled landscapes with fitness values drawn from Gaussian distributions with the appropriate mean and measurement variance of each genotype and counted the fraction of landscapes with a given number of maxima and minima (see app. A in the online edition of the *American Naturalist*). Figure 3 shows that the highest probabilities coincide with four and three maxima for CS I and CS II, respectively, consistent with the number of fitness maxima we identified on the basis of mean fitness (see fig. 2). Moreover, this analysis shows that the probability that the landscape is smooth with just a single maximum is below 10^{-7} for CS I and below 0.02 for CS II. Next, we compared these data with another set of resampled landscapes where

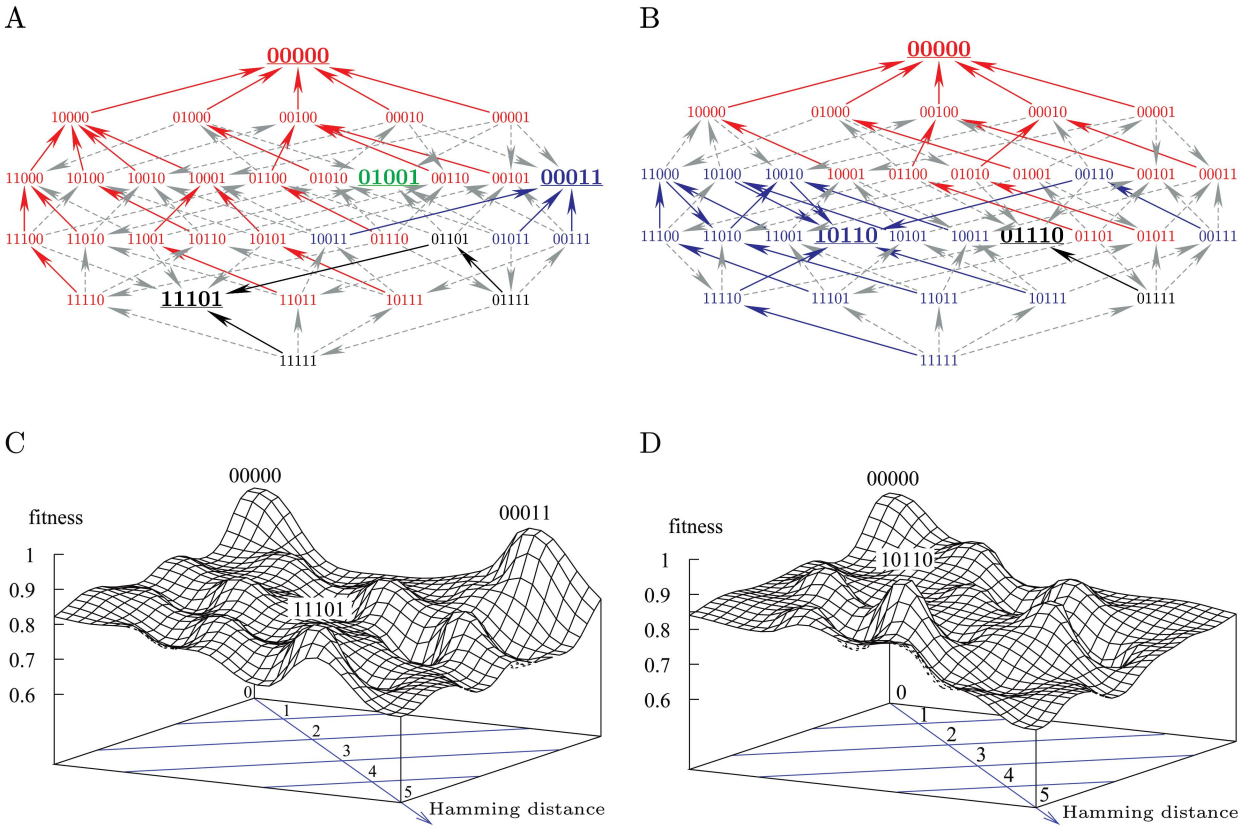

Figure 2: Fitness landscapes for both data sets. *A*, Arrow plot for CS I. Arrows indicate single-mutation steps directed toward the genotype with higher fitness. Genotypes corresponding to fitness maxima are shown in larger font size and underlined. The dashed gray arrows represent fitness-increasing steps that are not part of a greedy walk. Different colors indicate which genotypes are in the basin of attraction of the various fitness maxima; genotype 01001, shown in green, has only itself as a basin of attraction. *B*, Arrow plot for CS II. *C*, Rendering of the CS I fitness landscape as a two-dimensional surface. The genotypes were arranged in a diamond-shaped area that mimics the arrangement in the arrow plots in *A* and *B*, and the fitness values of the genotypes were interpolated by a smooth function. The most prominent fitness maxima are highlighted, and the lines in the base plane indicate the positions of genotypes with equal numbers of mutations. *D*, Two-dimensional surface plot for CS II.

there is no epistasis in the mean fitness. Specifically, we created 10^6 landscapes in which the mean fitness of a strain with n mutations is equal to $1 + \alpha n$, where $\alpha < 0$ is the average fitness effect of individual mutations estimated by the linear regressions shown in figure 1*A*, 1*B* and a constant measurement variance of 0.02 (corresponding to the average measurement variance of the experimental data) is assumed. This data set shows a radically different probability distribution of multiple fitness maxima (see fig. 3); in particular, the probability that more than one fitness maximum is present is now very small (i.e., <0.03). We also used the resampled landscapes to calculate the probability that the genotypes we had identified indeed represented fitness maxima and found that this probability was $\gtrsim$0.5 for each of the maxima (see fig. A1 in the online edition of the *American Naturalist*). Both analyses therefore provide support for the ruggedness of these fitness landscapes due to sign epistasis among the mutations involved leading to local fitness maxima and minima.

Asexual Adaptation on the Empirical Landscapes Is Constrained

Next, we consider the consequences of these empirical fitness landscapes for the problem of the evolution of sex by exploring the constraints experienced by sexual and asexual populations adapting on these landscapes. For this, we make two important assumptions. First, we assume that adaptation happens by the transition (by mutation and/or recombination) from one to another of the 32 genotypes, that is, by substitutions of wild-type or mutant alleles at the five loci only. Second, we assume here that all fitness differences among strains are real, and we deal with the consequences of the statistical uncertainties for

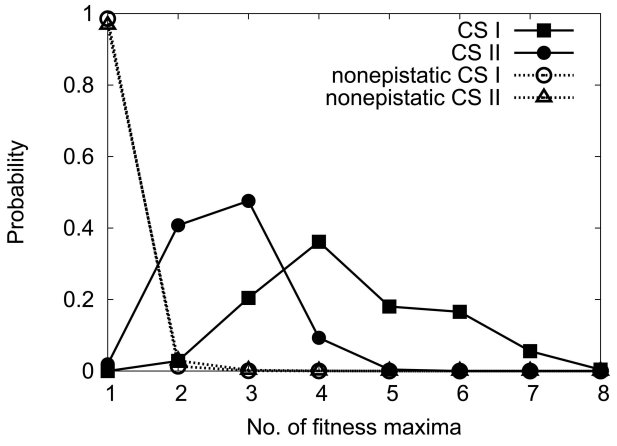

Figure 3: Probability distributions of the number of fitness maxima for both empirical landscapes based on resampling at least 10^6 sets of 32 fitnesses, using the appropriate mean and variance (assuming a Gaussian distribution) for each genotype. For the mean fitness of each strain, either the observed mean was used (CS I, *solid line, squares*; CS II, *solid line, filled circles*) or the expected mean in the absence of epistasis, which is equal to $1 + \alpha n$, where $\alpha < 0$ is the coefficient of linear regression and n the number of mutations, was used (CS I, *dotted line, open circles*; CS II, *dotted line, triangles*). The probability that these fitness landscapes contain multiple fitness maxima reduces from >0.98 to <0.03 when epistasis is removed from the fitness data.

the adaptive dynamics in appendix B in the online edition of the *American Naturalist*. There, we present the results of simulations based on fitness sets resampled from the original data, with the appropriate mean and variance for each genotype.

We studied the adaptive dynamics of asexual populations for a wide range of parameter values. In the strong selection–weak mutation (SSWM) limit ($NU\ln N \ll 1$ and $Ns \gg 1$, with s denoting a typical selection coefficient), where clonal interference (Gerrish and Lenski 1998; Park and Krug 2007) is unimportant, adaptation of an asexual population can be approximated by an adaptive walk on the landscape. Here we will determine the probability that the adaptive walk will arrive at one of the local maxima when it starts from the quintuple mutant. To this end, we need to specify the probability that a population located at a sequence σ fixes one of its mutational neighbors, which reads (Orr 2002)

$$\Pr(\sigma \to \sigma') = \frac{\pi(\sigma';\ \sigma)}{\sum_{\sigma''} \pi(\sigma'';\ \sigma)}. \tag{6}$$

Here $\pi(\sigma';\ \sigma)$ is the fixation probability of genotype σ' introduced as a single copy into a population of genotype σ, and the denominator sums these probabilities over the five nearest (i.e., with Hamming distance 1) neighbors of the genotype σ. In the context of the Wright-Fisher model, $\pi(\sigma';\ \sigma)$ can be approximated as $\pi \approx 2s$ when the selection coefficient $s = w(\sigma')/w(\sigma) - 1$ is small and positive. We neglect the fixation probability of deleterious mutations. In the actual calculation we numerically solve the implicit equation $1 - \pi = \exp[-(1 + s)\pi]$ following Haldane (1927), which is known to provide an accurate approximation for the exact fixation probability of the Wright-Fisher model (Barrett et al. 2006). Then we enumerate all fitness-increasing paths and assign a probability to each path by using the transition probabilities from equation (6). Although the analysis is generally applicable to other initial conditions, we give the results only for the initial condition $\sigma = (11111)$.

The simulation results for asexual populations adapting under the SSWM regime ($U = 10^{-8}$ and $N = 10^5$) on both landscapes are shown in figure 4 and table 2. Clearly, most adaptive walks end at a local maximum, and few reach the global maximum on both landscapes. The populations stay monomorphic most of the time, alternated by brief periods of polymorphism during jumps to another peak. Across 10,000 runs, mean fitness approaches asymptotic values of 0.90 and 0.94 for CS I and CS II, respectively. These asymptotic values are consistent with predictions from the adaptive walk scenario.

Beyond the SSWM regime, there are two conspicuous regimes of asexual adaptation that depend on the population size N and mutation rate U (Jain and Krug 2007). At very large N, the population behaves as in the infinite population limit, and the evolutionary dynamics are completely deterministic. In this regime all possible genotypes are simultaneously present in the population (but most of them at extremely small frequencies). A simple algorithm exists to predict the trajectories of the most populated genotype that a population will follow from an arbitrary starting point to the global fitness maximum (Jain and Krug 2005; Jain 2007); see appendix C in the online edition of the *American Naturalist*. For our empirical landscapes these trajectories are for CS I and CS II, respectively,

$$11111 \to 11101 \to 00011 \to 00000, \tag{7}$$

$$11111 \to 11110 \to 10110 \to 00000. \tag{8}$$

Apart from the first step in trajectory (8), all intermediate genotypes appearing in these trajectories are local fitness maxima. We find that the deterministic regime is reached in our landscapes for population sizes exceeding $N \propto 1/U^2$.

Between the SSWM and the deterministic regime lies the locally deterministic regime of Jain and Krug (2007), where the population behaves deterministically up to a crossover time T_x when the first local maximum is reached.

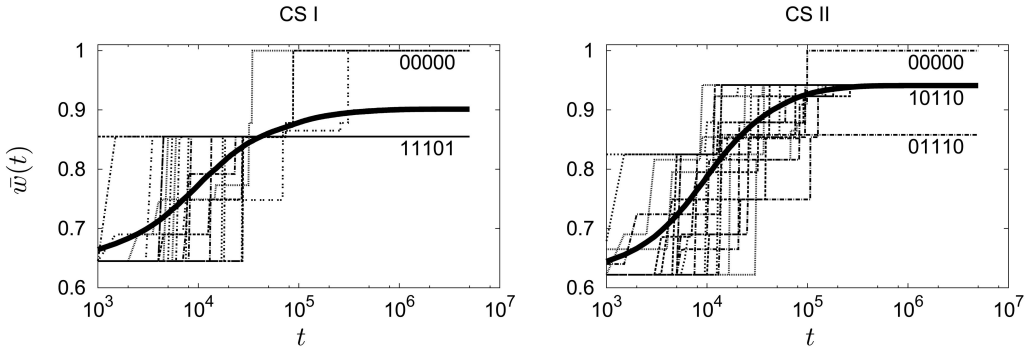

Figure 4: Twenty sample adaptive walks starting with genotype 11111 on two empirical landscapes in the strong selection–weak mutation regime ($N = 10^5$ and $U = 10^{-8}$). On the CS I landscape, 17 adaptive walks arrived at local maximum 11101, and only three walks reached the global maximum. The probability of arriving at two other local optima (01001 and 00011) is so low that no walk among 20 samples reached them (see table 2). On the CS II landscape, 18 adaptive walks reached local maximum 10110, and only one walk reached 00000 or the other local maximum 01110. The curves are the average of 10,000 independent adaptive walks; the proportions of walks ending at each fitness maximum are given in table 2.

This initial stage of adaptation can be described as a "greedy" adaptive walk that always chooses the nearest-neighbor genotype of highest fitness and gets stuck at local maxima. For example, a greedy walk on landscape CS II starting from the sequence 11111 will take the steps 11111 → 11110 → 10110, and on the CS I landscape, the walk becomes 11111 → 11101. Beyond the initial stage, the behavior is determined by the stochastic escape from local maxima by the creation of multiple mutants (Weinreich and Chao 2005). Provided that the mutation rate is sufficiently small, the timescale for the escape dynamics is well separated from the greedy walk phase. For larger populations the escape dynamics becomes deterministic as well, taking the form of an "adaptive flight" between local maxima, as illustrated in trajectories (7) and (8).

In the following, the intermediate regime will be referred to as the greedy walk regime. It sets in when the number NU of mutants produced in one generation exceeds the number L of one-mutant neighbors of a given genotype. It should, however, be noted that the boundaries between the different regimes are not sharply defined and may depend on the details of the landscape (Jain and Krug 2007). The greedy walk concept allows us to assign to each local maximum a basin of attraction that contains all genotypes that end up at this maximum under the greedy walk dynamics. The basins of attraction for both fitness landscapes are illustrated in color in the arrow plots of figure 2.

Simulating the Effect of Sex on the Empirical Landscapes

Given the adaptive constraints for asexuals, we ask whether sex and recombination can be beneficial for adaptation on these landscapes. As a general first question, we ask whether recombination among locally optimal genotypes is beneficial in the sense that it will create either the globally most-fit genotype (i.e., the wild type) or a genotype that is part of the basin of attraction of the global optimum. In CS I, three locally optimal genotypes exist, but recombination among them cannot create the globally optimal wild type (table 3). Similarly, recombination between the two locally optimal genotypes of CS II does not create the high-fitness wild type. Rather, recombination has a direct negative effect on offspring fitness on both landscapes. Although sex cannot generate the global optimum directly, it can produce genotypes with access to the global optima via mutation and selection, that is, that appear in the basin

Table 2: Summary of the asexual adaptive walks for the strong selection–weak mutation regime on both landscapes

Maximum	Adaptive walk weight[a]
CS I:	
00000	.294
00011	.033
11101	.672
01001	.001
CS II:	
00000	.134
10110	.757
01110	.109

[a] Shown are the probabilities that the adaptive walk that starts at the sequence 11111 will end up at one of the fitness maxima that are present (see table 1). The wild-type genotype (00000) represents the global maximum in both landscapes; the others are local maxima.

Table 3: Recombination among locally optimal genotypes on both *Aspergillus niger* adaptive landscapes

Parents	Offspring[a]	Effect on mean fitness[b]
CS I:		
01001 × 00011	00001, 01001, 00011, 01011	−.096
01001 × 11101	01001, 11001, 01101, 11101	−.015
00011 × 11101	00001, 10001, 01001, 00101, 00011, 11001, 10101, 10011, 01101, 01011, 00111, 11101, 11011, 10111, 01111, 11111	−.145*
CS II:		
10110 × 01110	00110, 10110, 01110, 11110	−.030

[a] Genotypes that are part of the basin of attraction of the globally optimal wild type are underlined (shown in black in fig. 2*A*, 2*B*).

[b] Mean offspring fitness − mean parental fitness, tested with one-sample, two-tailed *t*-test.

* $P < .001$.

of attraction of the global optimum (table 3). Thus, in a polymorphic population with genotypes occupying local fitness maxima, recombination can release populations that are stuck on local fitness peaks and allow them to evolve toward the global optimum, although this depends on the landscape's topography. However, it is not clear which conditions would lead to a polymorphic population occupying several fitness maxima.

In the following we therefore use simulations to ask whether and when sex provides an adaptive advantage for a population that initially consists of the low-fitness quintuple mutant on these landscapes. In the SSWM regime, the population remains monomorphic most of the time, and only during the selective sweep are there two segregating sequences (whose Hamming distance is 1 within our mutation scheme). Hence, in this regime, recombination cannot play any role, and the adaptive dynamics are the same for sexual and asexual populations. For example, we simulated a sexual population with $r = 1$ and 0.01 using the same parameters as in figure 4, and we observed the same dynamics as for the asexual population (data not shown).

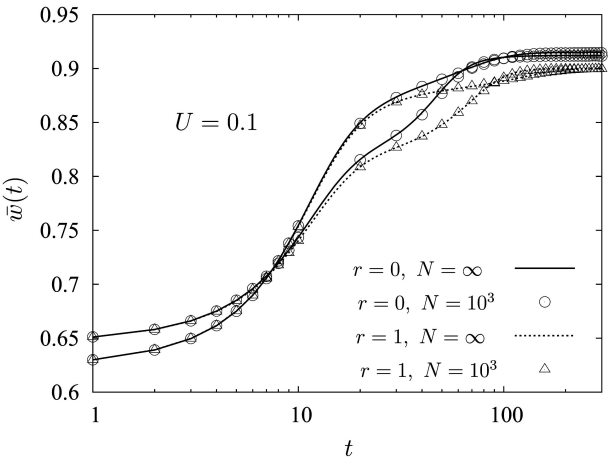

Figure 5: Effect of sex during adaptation on empirical fitness landscapes for high mutation rates ($U = 10^{-1}$). Shown are semilogarithmic plots of mean fitness as a function of time (in generations) for 10,000 independent runs with ($r = 1$) or without ($r = 0$) recombination. Simulation results for finite ($N = 10^3$, *symbols*) and infinite (*lines*) population size are indistinguishable. The data sets starting at fitness 0.65 are results for the CS I landscape; those starting at lower fitness are for the CS II landscape. The mutational load (i.e., the difference between equilibrium mean fitness of the asexual population and 1) and the recombinational load (i.e., the difference between equilibrium mean fitness of the sexual and asexual populations) do not strongly depend on the particular landscape.

Infinite Population Size. When the population size is large enough, the population can be polymorphic, and recombination may play a role. Let us first investigate the infinite population limit where the dynamics are deterministic. The stochastic simulation can then be replaced by simply iterating the mutation-selection recombination equation (5) with equations (1)–(3) for the population frequencies. Referring to the asexual trajectories (7) and (8), we expect that the adaptive dynamics on the CS II landscape are simpler, in that only a single intermediate fitness maximum is involved. We therefore begin with the analysis of CS II.

As figures 5 and 6 show, recombination on this landscape never confers an advantage; rather, sex is always deleterious. The disadvantage of sex is particularly dramatic for $r \geq 10^{-1}$, where recombination is seen to prevent the escape of the population from the local maximum 10110. The suppression of the escape from a local fitness peak due to recombination is well known from studies of two-locus models (Eshel and Feldman 1970; Michalakis and Slatkin 1996; Phillips 1996; Weinreich and Chao 2005). Qualitatively, the suppression is due to the recombination of escape genotypes such as 10000, 00100, and 00010, which are at Hamming distance 2 from the local peak and at Hamming distance 1 from the wild type, with the (dominant) local peak genotype 10110. Because of relation (4), such an event produces offspring closer to

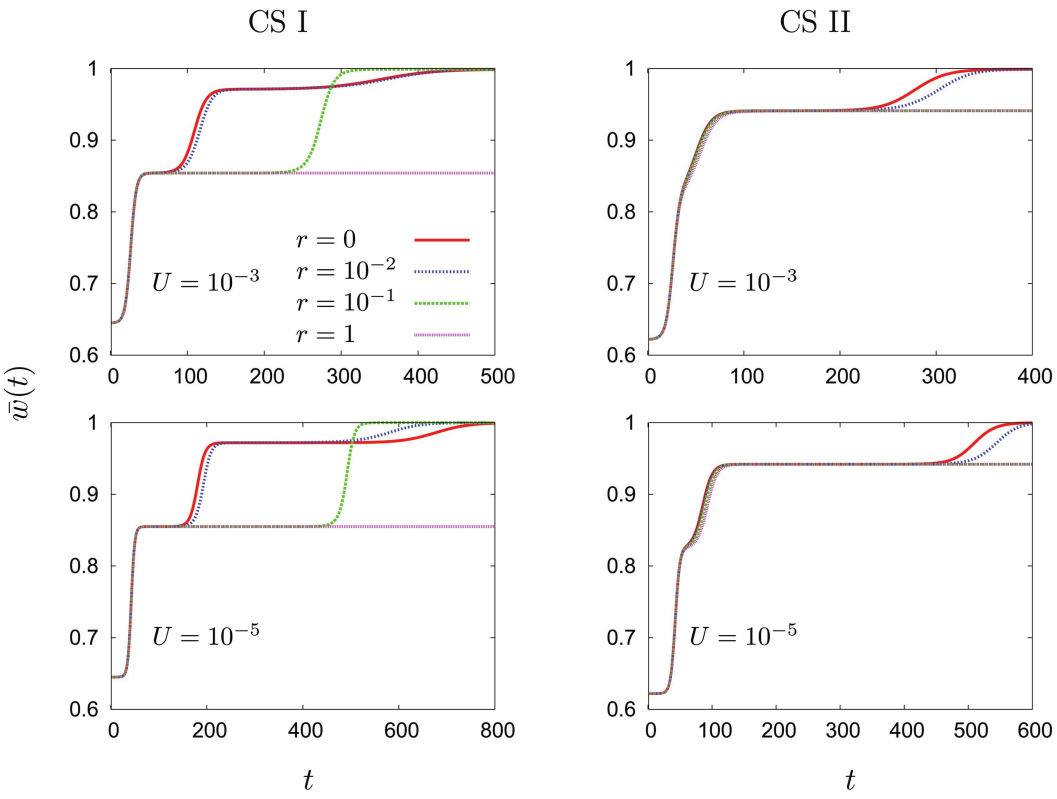

Figure 6: Infinite population dynamics during adaptation on two empirical fitness landscapes, CS I (*left*) and CS II (*right*), with and without recombination and $U < 10^{-1}$. For CS I, sex is initially deleterious, but low rates of recombination (r) later become advantageous during the escape from the first local optimum (genotype 11101 with fitness 0.855), where the second optimum (00011 with fitness 0.972) is bypassed when $r = 10^{-1}$. For CS II, recombination is always disadvantageous.

the local peak, thus pushing the population away from the wild type.

For a simple quantitative estimate of this effect, we resort to the standard two-locus setting with an initial genotype *ab* of fitness 1, a double mutant *AB* of fitness $1 + s$, and low–fitness valley genotypes *aB* and *Ab* (Crow and Kimura 1965). When a double mutant *AB* appears, it survives recombination with the dominant genotype *ab* with probability

$$W_{AB|\,ab,\,AB} + W_{AB|\,AB,\,ab} = 1 - \frac{r}{2} \tag{9}$$

according to equation (3). Provided that it survives, it will increase in frequency by a factor $1 + s$. On average, the mutant therefore increases in the population if $(1 - r/2)(1 + s) > 1$ or

$$r < r_c = \frac{2s}{1 + s}. \tag{10}$$

For the CS II landscape $s \approx 0.06$ and $r_c \approx 0.12$, in reasonable agreement with the numerically observed behavior (but note that in CS II the Hamming distance between the two fitness peaks is actually 3). The complete suppression of escape observed for $r \geq 0.1$ is likely related to the appearance of multiple stationary solutions of the deterministic evolution equations, with one solution localized at the local fitness peak and one localized at the wild type; see Higgs (1998) for a discussion of this phenomenon in a two-locus model with peaks of equal height. A detailed analysis of the two-locus problem with two unequal peaks shows that $r > r_c$ is a necessary (but not sufficient) condition for the appearance of multiple solutions (S.-C. Park and J. Krug, unpublished manuscript).

The infinite population dynamics on landscape CS I are slightly more complicated. When $U \geq 10^{-1}$, recombination is always deleterious because a recombinational load (as well as the mutation load) is observable, similar to landscape CS II (fig. 5). However, when $U \leq 10^{-3}$, sex appears advantageous for some recombination rates because the

time to arrive at the globally optimal genotype is shorter than that for the asexual population, although the time to escape from the local maximum (11101) is longer than that for the asexual population (fig. 6). Closer inspection of this case reveals that recombination allows the population to bypass the intermediate maximum 00011,[2] but this mechanism is operative only in a narrow range (e.g., the range $0.08 \lesssim r \lesssim 0.12$ or $r \lesssim 0.03$ for $U = 10^{-5}$; data not shown). This implies that the escape time is a nonmonotonic function of r in this regime, with a well-tuned rate of recombination slightly accelerating the adaptation process; similar effects have been observed in two-locus simulations (Weinreich and Chao 2005). Extensive simulations on resampled landscapes reported in appendix B show that the existence of an "optimal" recombination rate near which sex is generally advantageous is a robust feature of the CS I landscape, which persists when measurement errors are taken into account.

Finite Population Size. For finite N, we simulated 10,000 independent runs for each parameter set. For $U = 10^{-1}$, the simulation results with $N = 10^3$ are already indistinguishable from the infinite population dynamics for both landscapes (fig. 5). We also simulated smaller population sizes ($N = 10$, 100) and observed qualitatively similar behavior (data not shown). We can therefore conclude that recombination is not advantageous on these empirical landscapes for any population size when the mutation rate is high.

The situation becomes slightly more complicated at intermediate population size for lower mutation rates. In the intermediate regime, where the dynamics are indistinguishable from a deterministic greedy walk up to the time at which a local maximum is reached (i.e., up to T_x), sex is generally deleterious on landscape CS II. There is only a small advantage of recombination during the initial greedy walk stage where the dynamics are slightly different from deterministic dynamics, but during the final escape stage, recombination is still deleterious (data not shown). This slight advantage of sex during the early stages of adaptation is insignificant for long-term adaptation, which is governed by the time needed to move from a local peak to the global peak.

As expected from the infinite population dynamics, landscape CS I also shows more complex adaptive behavior for finite populations (fig. 7). Let us begin with the initial

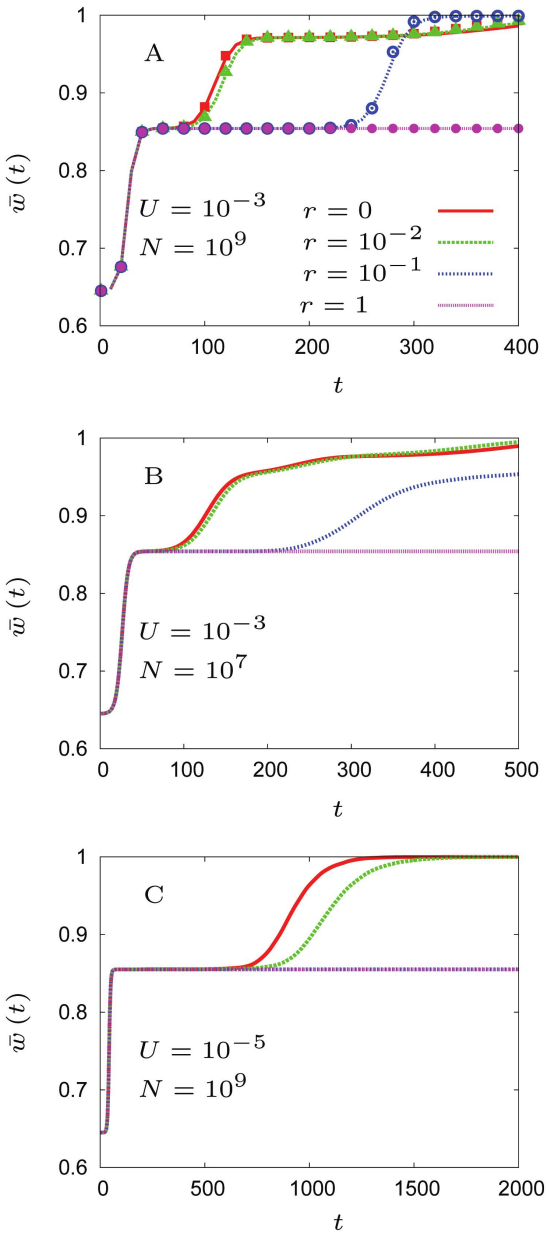

Figure 7: Adaptive dynamics for CS I for three different parameter sets (mean fitness trajectories of 10,000 independent runs). *A*, Adaptive dynamics of the finite populations (*symbols*) resemble the deterministic behavior (*lines*). For $r = 10^{-1}$, the population bypasses the second local optimum (00011 with fitness 0.972). Bypassing as a result of recombination does not happen when either N (*B*) or U (*C*) is lower, although there is a slight advantage of sex in the escape stage for $r = 10^{-2}$ for the conditions of *B*; the lines in *B* and *C* show the finite population results.

greedy walk stage. Because the attractor of the quintuple mutant, i.e., the local maximum 11101, is only one mutation away, we do not expect any difference between the asexual greedy walks and the sexual greedy walks. That is,

[2] Note that local maximum 11101 is at the same mutational distance ($d = 4$) from both the intermediate maximum 00011 and the global maximum 00000. The reason for the detour performed by the asexual trajectory (7) is that the initial point 11111 is closer to 00011 than to the global maximum (Jain and Krug 2005); indeed, an infinite asexual population starting from 11101 moves directly to the global maximum without visiting 00011 (data not shown).

up to the time when fitness reaches the value 0.858, one cannot see any difference in the rate of adaptation between sexual and asexual populations. Hence, the escape stage will be of main interest for landscape CS I. For large populations, adaptation is governed by deterministic dynamics (see fig. 7*A*). It appears that the escape advantage seen at intermediate r (fig. 7*A*) depends on a sufficiently large mutation supply rate: when either N (fig. 7*B*) or U (fig. 7*C*) is lower, this benefit of recombination disappears. The fact that we find the escape advantage of recombination only on landscape CS I indicates that it depends on the topographical details of the fitness landscape.

Discussion

While epistasis includes all possible forms of deviation from independent gene effects, students of the evolution of sex have been interested mostly in a single particular form of magnitude epistasis, that is, negative epistasis (Kondrashov 1993; Otto and Lenormand 2002; de Visser and Elena 2007; Kouyos et al. 2007). Negative epistasis is one possible source of negative linkage disequilibrium and, hence, of a long-term advantage of sex by increasing the genetic variation in fitness and accelerating adaptation; alternatively, genetic drift combined with directional selection can cause negative linkage disequilibrium. Despite increasing efforts to detect negative epistasis in recent years, the support for this form of epistasis is weak (de Visser and Elena 2007; Kouyos et al. 2007). However, other forms of epistasis may be relevant for the problem of the evolution of sex as well. For instance, sign epistasis, causing variation in the sign of the fitness effect of mutations across genotypes, may impose adaptive constraints caused by valleys of low fitness separating fitness maxima (Weinreich and Chao 2005; Weinreich et al. 2005). Whether and when recombination may facilitate adaptation on such rugged fitness landscapes is largely unknown and depends in part on the topography of the fitness landscape (i.e., the number, height, and distance of fitness peaks).

In this study, we have analyzed fitness data from two sets of 32 strains of the fungus *Aspergillus niger* carrying all possible combinations of five mutations with individually deleterious effects. In a previous study (de Visser et al. 1997), we used these strains to detect magnitude epistasis by studying the overall relationship between mean fitness and mutation number. We found no support for prevailing negative or positive epistasis, despite significant magnitude epistasis of both signs for particular pairs of mutations. Our new analyses were aimed at detecting sign epistasis and describing the fitness landscapes for these sets of five loci. We found that among the 128 unique single-mutation steps present in the two sets combined, 38 caused fitness increases, despite their individually deleterious effects in the wild type. In addition, when we compared the fitness of each strain with that of its five single-mutation neighbors, we found several statistically supported fitness maxima and minima in both sets of strains. These fitness maxima and minima indicate ruggedness of the fitness landscapes resulting from sign epistasis.

We then used simulations with the Wright-Fisher model to study whether and when recombination might facilitate adaptation on these empirical fitness landscapes. We first found that adaptation was constrained for asexual populations because only a minority of adaptive walks of finite populations from the low-fitness quintuple mutants ended at the global fitness optimum (see fig. 4) and because even infinitely large populations adapt by sequentially visiting local maxima (eqq. [7], [8]). The simulations showed that adaptation on these rugged landscapes generally involved two distinct stages: the approach to a local optimum and the escape from a local optimum to a higher optimum.

Two general conclusions can be drawn from the simulations comparing asexual and sexual populations. First, recombination may provide a small benefit during the approach to a local optimum at intermediate N and U if the local optimum is more than one mutation removed from the ancestral genotype. This small benefit is probably due to the fact that the alleles involved in the local optimal genotype are in negative linkage disequilibrium due to drift and selection, causing recombination to combine them more often than to disrupt them (Fisher 1930; Muller 1932). This effect becomes more pronounced as the distance to the global peak increases. We have verified in simulations that recombination results in a significant speedup of adaptation in a five-locus system without epistatic interactions (data not shown; for similar results see Kondrashov and Kondrashov 2001; Kim and Orr 2005). However, this small advantage is generally insignificant for long-term adaptation, which is limited by the time needed to escape from a local peak.

Second and more important, during the escape stage the effect of recombination is generally deleterious and increases the time to escape from the local optimum. At high recombination rates ($r = 1$), we observed only deleterious effects, which are caused by the net breakdown of escape genotypes that arise by mutation. We also found some conditions where sex and recombination could facilitate the population's escape. The fact that we observed benefits of recombination for only one landscape indicates that the landscape's specific topography is involved in this benefit. Also, the escape benefit is apparent only when N and U are sufficiently high (see fig. 7), consistent with the dependence of any advantage of recombination on a sufficiently polymorphic population. In a more general sense, the effect of recombination during the escape stage will

depend on conditions where the alleles necessary for the escape genotype are present in negative linkage disequilibrium in genotypes with relatively high fitness.

Some theoretical studies have considered the effect of sex and recombination on multilocus fitness landscapes with varying degrees of ruggedness. Otto et al. (1994) found that recombination slows the crossing of a single wide fitness valley in a system with $L = 20$ loci but speeds up the ascent to the peak if the population is initialized on the hillside. Kondrashov and Kondrashov (2001) found a general disadvantage of sex in a particular two-dimensional landscape with a single "smooth" ridge toward high fitness and no local fitness maxima. Later, Watson and Wakeley (2005) modified this landscape and found conditions where sex does provide an advantage. The landscapes considered in these articles are similar to ours in that most paths to the global optimum are inaccessible to adaptive evolution, but they differ in that they contain extended neutral networks instead of local fitness peaks. On the other hand, Hadany and Beker (2003) found a general advantage of recombination in simulations of rather small populations in fitness landscapes generated according to Kauffman's (1993) NK model. Recombination in NK landscapes was also considered by Bergman et al. (1995), who were, however, interested mainly in the effects of population dispersal. Together with the work presented here, these fragmentary results suggest that the effects of recombination depend on features of the landscape topography that go beyond the mere presence or absence of sign epistasis. The precise nature of these features remains to be elucidated in future work.

How do we reconcile our finding of a general lack of sex benefit on rugged fitness landscapes with the several experimental reports (Rice 2002; de Visser and Elena 2007) of faster-evolving sexual populations compared with asexual populations? These reports suggest that the fitness landscapes involved have different topographies from ours; for example, they contain smooth areas or ridges allowing unconstrained adaptation. These differences could have a variety of causes. First, our landscapes are based on the interactions among mutations with an individually deleterious effect, which may differ systematically from those involving beneficial mutations. A similar study of the fitness landscape of TEM-1 β-lactamase involving five mutations with jointly beneficial effects also found sign epistasis and local ruggedness (Weinreich et al. 2006) but did not find the severe adaptive constraints imposed by isolated local fitness maxima that we found. We are unaware of other data sets that would allow a more systematic test of whether the nature of the mutations studied is responsible for the differences in landscape topographies. Second, sampling error from studying only a handful of loci may cause our landscape to "miss" smooth ridges or surfaces connecting local peaks, particularly if those are rare. Third, the topography of fitness landscapes may depend on the level of fitness. A study of protein fitness landscapes found a relatively smooth surface for low-fitness proteins and ruggedness above a certain fitness value (Hayashi et al. 2006). If ruggedness appears to be a typical feature of regions of relatively high fitness, our results suggest that sex would be beneficial only for low-fitness individuals, consistent with the negative correlation between fitness and recombination rates observed for many organisms (Hadany and Beker 2003).

Schoustra et al. (2007) recently observed a specific advantage for mitotic recombination in the face of sign epistasis in the homothallic fungus *Aspergillus nidulans*. By comparing adaptation in diploid and haploid strains, they found that four diploid strains that spontaneously reverted to haploidy gained the highest fitness. Backcrosses of these lines showed that multiple mutations were present, some of which had an individually deleterious effect. Therefore, these lines seemed to have accumulated recessive deleterious mutations when diploid that showed their combined beneficial effect due to sign epistasis in haploid recombinants produced in the parasexual cycle. The advantage of recombination in rugged fitness landscapes may thus depend on the relative length of the diploid and haploid phases of the sexual cycle, which affect the adaptive constraints experienced from fitness valleys.

In conclusion, further progress in our understanding of the costs and benefits of recombination will increasingly depend on the combination of experimental studies of the structure of real fitness landscapes with population-genetic simulations comparing different reproductive strategies. In this article we have attempted a modest first step in this direction.

Acknowledgments

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